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SIGNIFICANT CONTRIBUTIONS IN PLANETARY BIOLOGY FROM AMES RESEARCH CENTER 1974

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INTRODUCTION

The Planetary Biology Division has two major objectives: (1) To investigate the origin, evolution, adaptation and distribution of life and life-related molecules throughout the universe. (2) To search for and characterize extraterrestrial life at biological and molecular levels. Both basic and applied research are conducted within the Division in order to reach these goals.

The activities of the Division during 1974 included research done in direct support of space missions. The Gas Exchange Experiment was readied for the Viking mission of 1975. Plans were initiated for the gas chromatograph atmospheric analyzer of the Pioneer-Venus mission. Considerations were given to the effects of sterilization as related to the proposed Mars Surface Sample Return mission. Details of this research and of research indirectly related to missions is given on the following pages.

CHEMICAL EVOLUTION BRANCH

Research in the Chemical Evolution Branch continues to focus on chemical aspects of Planetary Biology. The ultimate goal of the work is to understand the chemical events leading to the origin and evolution of life in order to provide a sound scientific basis for the search for extraterrestrial life and related processes. To achieve this goal the program has been divided into six interrelated research areas each of which is directed to a part of the cosmos. These areas are concerned with the organic or carbon chemistry associated with:

1) planetary environments
2) interstellar medium
3) meteorites
4) Earth
5) Mars
6) Moon

Planetary environments

Studies on the effect of hydrogen (H_2) on the course of abiotic organic synthesis by high energy processes in planetary atmospheres have been initiated. Gamma radiolysis of methane-hydrogen gas mixtures shows

that unsaturated, low molecular weight hydrocarbons are produced only from pure methane and only in the early stage of reaction. No unsaturation is detectable in gas mixtures with methane-hydrogen ratios ranging from 0.5 to 0.0005. Furthermore the production of hydrocarbons larger than CH_4 drops off by a factor of 10^{-6} in going from 50% to 0.05% methane despite the fact that the initial methane concentration was reduced by only a factor of 10^{-3}. The profound influence on yields and nature of products exerted by hydrogen implies that models for organic synthesis on the hydrogen-rich outer planets require both reconsideration and re-examination. Organic synthesis in these planets may occur to much lower extents than previously estimated.

By means of theoretical methods, the nature, distribution and energy of the electrons have been determined for a series of organic compounds (amines, nitriles and amino nitriles) relevant to prebiotic and cosmochemistry. These compounds contain reactive sites which play important roles in their chemistry. Information about the distributions of electrons at the reactive sites leads to understanding of the mechanisms and pathways involved in reactions of these compounds and provides a basis for the prediction of the behavior of these compounds in the primordial million. The theoretical calculations support the view that carbodiimide (HNCNH) is the reactive form of cyanamide (H_2NCN) which promotes condensation reactions between amino acids, sugars and phosphate, and nucleotides.

Contributing Personnel: S. Chang, R. Mack, M. Lo; Collaborating Personnel: G. Loew, Stanford University

Interstellar medium

The implantation of low energy carbon and nitrogen ions into silicate particles leads to formation of carbon and nitrogen species which are chemically bound to the mineral matrix. When the implanted samples are heated to 600°C or higher the carbon and nitrogen are released as CO and N_2 respectively. In the interstellar dust clouds low energy ions could originate from stellar winds, supernova envelopes, shock waves and cloud penetration into galactic arms. Release of CO and N_2 from the grains into space could result from collisions of dust grains and/or ion sputtering. Therefore, much of the carbon found as CO, the most abundant carbon molecule in the interstellar medium, may have been produced by ion implantation followed by thermally induced expulsion from the dust grains.

In previous theoretical studies of the chemistry of hydrogen cyanide (HCN) the presence in the interstellar medium of methylene imine (H_2CNH) and methyl amine (H_3CNH_2) was predicted. Both of these molecules have now been discovered in the interstellar medium by radioastronomy.

Contributing Personnel: S. Chang, K. Lennon; Collaborating Personnel: G. Loew, Stanford University

Meteorties

More information has been gained concerning the amino acids of the Murchison meteorite. A search has been made for peptides. These

compounds have not yet been clearly identified, but, if present, they can account for at most only 6 to 9 percent of the total amino acids recovered by acid extraction methods. Newly developed procedures also show that certain amino acids precursors are present which are not basic chemically. The exact nature of the precursors is unknown.

Stereochemical studies of isovaline in the Murchison meteorite show that this compound occurs as a racemic mixture. Because isovaline has no hydrogen on its asymmetric carbon atom, it could not have undergone racemization by the usual mechanisms. Therefore, isovaline was originally synthesized as a racemic mixture and did not result from the racemization of one of its optical isomers. Thus, other amino acids were likely synthesized in the same manner. This work reaffirms that meteorite amino acids were synthesized abiotically in space, and the amino acids do not represent evidence for extraterrestrial life where one optical isomer would be expected to dominate.

An attempt was made to determine if the Fischer-Tropsch-Type (FTT) model could be used to account for the amino acids in meteorites as claimed by Anders. These attempts failed to produce significant concentrations of the compounds, and the usefulness of FTT model to explain amino acids in meteorites is questioned.

To determine the origin of amino acids in meteorites, the behavior of potential classes of precursors of amino acids under the conditions used to extract free amino acids from meteorites was investigated. It was estimated that less than 10 percent of the free glycine (the most

abundant free amino acid) could be derived from amides, dipeptides, nitriles, cyanuric acid and formaldehyde polymers.

Methylsuccinic acid occurs as a racemic mixture in the Murchison meteorite and also in the products from electric discharge experiments. In order to understand these observations relative to what occurs naturally on Earth, methylsuccinic acid was extracted from urine. In contrast to the compound extracted from the meteorite, only one optical isomer of urinary methylsuccinic acid was present. Thus, it can be assumed that dicarboxylic acids in meteorites did not arise from biological processes but rather were generated in an abiotic, extra-terrestrial synthesis as were the amino acids.

Contributing Personnel: J. Lawless, B. Zeitman, D. Brown, S. Chang, R. Mack, K. Kvenvolden, G. Pollock, S. Cronin, C-N. Cheng; Collaborating Personnel: J. Cronin, Arizona State University

Earth

The Earth provides a useful model for testing concepts and techniques to be applied in planetary exploration. Continued study of amino acids has given information relating to the problems of diagenesis, geochronology and paleothermometry. The fate of lysine in marine molluscs has been examined; besides pipecolic acid, lysine also produces, under simulated diagenetic conditions, delta-aminovaleric acid, alpha-aminoadipic acid and epsilon-aminocaproic acid. Hydroxy-proline was discovered to be one of the principal amino acids in wood,

and in the same samples proline was observed to racemize at a faster rate than other amino acids. The degree of racemization of amino acids in bones from early man in California was determined for sites at Stanford, Sunnyvale and Del Mar. The results show that amino acids in bones from Sunnyvale and Del Mar are more racemized than in bones from Stanford. The age of Stanford Man is 4000-5000 years. The other bones are considered to be older, perhaps 30,000 to 40,000 years, based on the amino acid racemization data. The degree of racemization of aspartic acid in human teeth from patients ranging in age from 11 to 65 years was found to increase with increasing age. Calculations predict that the average temperature experienced by these teeth is 35-36^{o}C which is close to the normal body temperature of 37^{o}C. This observation supports the idea that racemization of amino acids in bones follows first order reaction kinetics. Amino acids in fossil shells from marine terraces along the California coast were examined to determine if the degree of racemization could be used for correlative and chronologic purposes. Preliminary results show the first marine terrace can be correlated from Ano Nuevo to Newport Beach. The first terrace at Goleta, however, is younger.

A possible model has been tested for the origin of optical activity in the primitive Earth. The preferential adsorption of D- and L-amino acids on d- and l- quartz was investigated. A typical experiment

consisted of equilibrating D, L-alanine plus ^{14}C D-alanine with equal amounts of powdered d- and l-quartz. The difference in amount of ^{14}C D-alanine adsorbed by d- and l-quartz was measured by scintillation counting. The results showed that d-quartz preferentially adsorbs D-alanine. When ^{14}C L-alanine was used the opposite effect was observed; that is, l-quartz preferentially adsorbs L-alanine. The differential adsorption was about 7 percent. This work unequivocally demonstrates that optically active quartz preferentially binds amino acids, and the results suggest a possible route to the origin of optically active amino acids. Other possible routes to explain optical activity are under investigation and include parity violation in β-decay and artificially polarized β-rays.

To understand possible interactions among molecules in the context of the origin of life on Earth, studies by ultraviolet difference spectroscopy has shown an interaction at the monomer level between selected aromatic amino acids and nucleotides.

Two techniques for mass spectrometry have been considered. (1) A correlation has been found between semi-empirical molecular orbital calculations and mass spectral fragmentation patterns of a series of substituted acetophenozes. The findings may provide a theoretical basis for the prediction of mass spectral data. (2) Semi-empirical molecular orbital calculations have been used to understand the mechanistics of mass spectral decomposition of three amino acids (alanine, β-alanine and sacosine). These calculations have resulted in the prediction of pathways of ion formation.

Contributing Personnel: K. Kvenvolden, E. Peterson, J. Flores, J. Lawless, J. Link; Collaborating Personnel: J. Bada, Scripps Institution of Oceanography; J. Wehmiller, University of Delaware; W. Bonner, P. Kavasmaneck, G. Loew, Stanford University; J. Orenberg, San Francisco State University.

Mars

Possible effects of sterilization procedures on simulated returned Martian samples were evaluated. In this preliminary work consideration was given to the changes that take place in the amino acid composition in soil, meteorite, basalt, limonite and montmorillonite when these samples were heated to different temperatures for various time periods. The results of this work show that heat treatments alter the amino acid content and the degree of racemization; at temperature above 200^{o}C most scientifically useful information is lost. Even at lower temperatures changes take place. These observations led to the recommendation that returned Martian samples should not be sterilized, but every effort should be made to preserve the chemistry as it is on the Martian surface.

Contributing Personnel: G.E. Pollock, E. Peterson, C-N. Cheng, S. Cronin, J. Mazzurco, K.A. Kvenvolden; Collaborating Personnel: E. King, University of Houston

Moon

A synthesis of data obtained by analysis of lunar soil samples has led to the formulation of a model for lunar carbon chemistry which describes the abundance, distribution, and isotopic composition of carbon in soil grains as a function of solar wind irradiation, micrometeorite bombardments and mineralogy. Recent results of sulfur analyses show that the abundance, distribution and isotopic composition of this element in lunar soils are profoundly affected by its recycling into the lunar atmosphere by impac vaporization and preferential recondensation on metallic particles. The results of lunar studies have implications for understanding the chemistry of organogenic elements on extraterrestrial bodies which, because of lack of atmosphere and magnetic field, are directly exposed to bombardment by solar wind or micrometeorite particles.

Contributing Personnel: S. Chang, K. Lennon, R. Mack, G. Walberg; Collaborating Personnel: I. R. Kaplan, U.C.L.A; E. Gibson, Johnson Space Center

Future

The future program of the Chemical Evolution Branch will continue to emphasize cosmochemistry and geochemistry as these fields relate to the origin and evolution of life and the search for extraterrestrial life. The organic chemistry taking place in planetary atmospheres and between planetary atmospheres and surfaces will be investigated by simulations based on recent atmospheric models. Carbon isotopic

studies will be made of the intramolecular isotopic distribution of amino acids in meteorites, and the isotopic composition of carbon in ancient rocks of Greenland will be determined to attempt an understanding of the early carbon cycle on Earth. Additional classes of organic molecules will be sought in meteorites in order to expand the present chemical catalogues. The degree of racemization of amino acids in the most ancient stromatolites will be measured. Molecular orbital calculations will continue to be applied to mass spectral phenomena in order to develop clues for predicting ion fragmention patterns. These kinds of calculation will also be directed to understanding the most likely interactions of molecules involved in planetary atmosphere and interstellar organic chemistry.

BIOLOGICAL ADAPTATION BRANCH

Activities of the Branch have been directed toward three general areas: (1) simulation of some of the environmental parameters of other planets, and study of the adaptation which enables organisms to survive those conditions; (2) studies of some of the cellular processes which are essential for adaptation, with suggestions as to their origin or evolution; (3) provision of study reports and laboratory data for Headquarters and Project offices.

PLANETARY ENVIRONMENTS

Mars - The search for extraterrestrial life is currently focused on this planet, whose environment is characterized by an extremely low water content, low temperature, and small quantities of oxygen. Although there is little water in the martian atmosphere now, the photographs by Mariner 9 strongly suggest extensive water erosion at some time in the past. Large quantities of water would have dissolved salts from the rocks and soil, and the salts would then have increased in concentration as the water evaporated. The characteristics of any martian biota would have to include adaptation to all of these environmental factors. The best terrestrial models for martian organisms appear to be the halophilic bacteria, which tolerate high concentrations of salts.

The plans for the research program on halophilic bacteria include the isolation of halophilic enzymes to determine their molecular properties, understanding the mechanisms that limit growth in a saline environment, and determining how widespread these characteristics are among halophilic and other bacteria. During the year, an enzyme (citrate synthase) has been isolated in pure form from an extremely halophilic bacterium, and from one which is moderately halophilic but anaerobic. A comparison is being made of the properties of the enzyme, which is important in carbon metabolism and energy generation, from the two organisms. In addition, it was found that the amount of this enzyme in the bacteria depends on the type of sugar on which they were grown; this led to the finding that the three sugars - glucose, galactose, and lactose - are metabolized by different pathways.

Contributing Personnel: L. I. Hochstein, M. J. Stevenson, R. Alico (NAS Associate), G. Tomlinson (Santa Clara University), G. Kritikos (San Jose State University), J. Fujimori (University of California, Berkeley).

Additional environmental stresses for organisms on Mars or the other outer planets are the low temperature and the low concentration of oxygen. Thus, further knowledge about organisms which are anaerobic psychrophilic halophiles is essential for predictions about finding native life, or the survival of terrestrial life. Conditions of that type would be encountered on Mars, Titan, and the Galilean satellites of Jupiter, and in the atmosphere of Jupiter and Saturn. In the laboratory, attempts are underway to isolate organisms capable of growth anaerobically, in high salt concentration, at near 0°C. Results suggest that organisms which can grow on a simple medium under those conditions are quite rare; studies are being extended to more complex media.

Contributing Personnel: P. H. Deal, H. M. Mack; M. Povse and T. Kraynick (Student Space Biology Program).

Jupiter - Ammonia is known to be present in the atmosphere of Jupiter in the approximate zone which is otherwise most favorable for life. It is also probably present on the other giant planets, Saturn, Uranus, and Neptune. Biology on these worlds would therefore be confronted with a relatively high concentration of ammonia, and a high pH. A major effort has been directed

toward exploring ammonia tolerance in a variety of bacteria, both pure laboratory strains and those isolated from alkaline and neutral environments in nature. Most of the organisms tested are extremely sensitive to ammonia at a high pH, where it occurs principally in the un-ionized form, NH_3. At lower pH, where ammonia is present as the ammonium ion NH_4^+, sensitivity is greatly reduced. Bacterial spores, on the other hand, are much less sensitive to un-ionized ammonia, even at pH 11 or above, than are their vegetative counterparts. Preliminary experiments with soil indicate that some organisms, possibly in the spore form, are able to survive for long periods when the soil is saturated with ammonia solution at pH 12. Further work will determine whether the survivors are inherently resistant or are protected from ammonia by the soil, perhaps by chemical binding, neutralization, or providing a barrier to penetration.

Contributing Personnel: P. H. Deal, H. M. Mack, K. A. Souza, L. Briggs (San Jose State University, M.A. degree research).

One of the unusually alkali-tolerant bacteria isolated from soil has been studied in some detail. It is a new species of the genus Flavobacterium, and has been named acidophobus; it grows over the pH range 8.0 to 11.4, with an optimum at pH 9.5.

When the intracellular pH was determined by several methods, it was found to be only 7.5 - not very different from that of some common bacteria (6.8-6.9). In addition, the optimum pH for an intracellular enzyme (malic dehydrogenase) from the alkalophilic organism was between 8.0 and 8.5, which is not significantly different from the optimum pH for the same enzyme in other bacteria. These results indicate that this organism creates an intracellular environment much different from the external environment. The mechanism of this control, and the reasons for lack of growth at an external pH similar to its internal pH, are questions still under study. In addition, other intracellular and membrane-bound enzymes, and external proteins (e.g. flagellin), will be compared to determine if the proteins more directly exposed to alkali have unique properties.

Contributing Personnel: K. A. Souza, T. L. Kirkman (New Mexico State University Co-op student), P. H. Deal, H. M. Mack.

Venus - There has been no serious consideration of a biota on the surface of Venus since the discovery of its high temperature. The atmosphere, however, has been suggested as a possible habitat for microorganisms, because it is dense, turbulent, and cooler. The bacteria which have been studied as models for that

environment are the thermophiles, which are found in nature at temperatures as high as boiling water. The synthesis of a thermostable membrane is obviously one of the critical problems confronting organisms growing at high temperature, especially since there is a high percentage of lipid in the membrane. It was shown here that as the growth temperature of the thermophile Bacillus stearothermophilus is increased, the average melting point of its membrane fatty acids also increases. This is also true for a variety of organisms which grow at low and moderate temperatures. Electron Spin Resonance (ESR) was then used to study the structure of the membrane in more detail. The results indicate that B. stearothermophilus alters its lipid composition in response to temperature, in order to maintain a balance between fluid lipids and gel lipids in the membrane at the temperature of growth. This suggests that the maximum and minimum growth temperatures of the organism may be determined by its biochemical ability to synthesize the lipids needed to maintain the membrane fluid-gel matrix. Two additional methods, fluorometry and differential scanning calorimetry, are now being used to study membrane structure. Preliminary results with the fluorescent probe N-phenyl-1-naphthylamine, which localizes in the membrane, support the

previous findings that the transition temperature of the membrane lipids is dependent on the growth temperature. Calorimetry studies, now underway, will measure overall transitions in the membrane.

Contributing Personnel: K. A. Souza, A. F. Esser (California State University, Fullerton), L. L. Kostiw, T. L. Kirkman (New Mexico State University Co-op student).

CELLULAR PROCESSES

Utilization of Light Energy - A novel form of energy conversion, found in the purple membranes of certain halophilic bacteria, is being studied. These bacteria are able to convert the energy of light into electrical energy, which is later used to synthesize ATP, but do not use the nearly universal chlorophyll photosynthesis system. It was established that a molecule of bacteriorhodopsin absorbs a light photon and undergoes a photochemical reaction cycle. At least four of the intermediates in the cycle have been characterized; they have lifetimes of between one hundredth and one millionth of a second. At one point in the cycle there is a release of a proton (H^+), and at another point there is a proton uptake. Study of the kinetics of these rapid reactions has identified the reactions at which the proton

exchanges occur. It is suggested that these are the key to the functioning of the purple membrane, for if the proton exchanges took place on opposite sides of the membrane, it would result in the actual translocation of protons and the generation of an electrical potential. This potential is then used to synthesize the energy currency (ATP) by reactions which are not yet understood. Resonance Raman spectroscopy has identified the group in the bacteriorhodopsin molecule which is the proton donor. The above results, obtained with isolated purple membrane or its components, have been verified by studies on whole cells. Measurements of the proton translocations induced by light in cell suspensions revealed a complex process, probably including the purple membrane proton pump and an incompletely understood light-induced change in membrane permeability. By activating the system with light of different wavelengths, it has been confirmed with cell suspensions that bacteriorhodopsin is the mediating pigment. Another interesting observation with whole cells is that when they are illuminated, respiration stops. It appears that they are capable of switching from the use of food for energy to light energy when it is available.

Photosynthesis provides essentially the only process by which light energy from the ultimate source, the Sun, is transformed into useable chemical energy on Earth. Presumably, other

planets will also be dependent on sunlight, but there is no assurance that life forms there would have evolved the terrestrial type of photosynthesis involving chlorophylls. The purple membrane provides the first biological system which uses light energy without using the photosynthetic pathway. As such, it is an extremely valuable example of alternative systems which may function on other planets. This work is proceeding at Ames under the direction of NASA Life Scientist Dr. Walther Stoeckenius, University of California, San Francisco.

Contributing Personnel: W. Stoeckenius, R. A. Bogomolni, R. Lozier, R. Baker.

Collaborating Personnel: J. K. Lanyi (Ames Research Center), W. W. Parson (University of Washington), A. Lewis (Cornell University), R. Cone (Johns Hopkins University).

Membrane Structure and Function - Membranes, comprised of lipids and proteins, not only bound the cell and regulate the transfer of material in and out, but also support and organize many of the enzyme systems inside the cell. The membranes of halophilic bacteria are of interest because they provide an opportunity to correlate the unusual chemical nature of the

lipids of these organisms with the salt-dependent properties of the whole cell envelope membranes. Extensive studies have been carried out with artificial membranes prepared from the lipids of extremely halophilic bacteria, using the techniques of Electron Spin Resonance (ESR), fluorometry, and dilatometry (volume changes with temperature). The results reveal that the extensive methyl branching of the lipid hydrocarbon tails causes the lipids to behave as if made from rigid segments, rather than displaying the usual highly flexible structure. The inclusion of squalene, a non-ionic lipid, caused tighter packing in the central, hydrocarbon region of the membranes, but increased the spacing of the lipid headgroups at the membrane surface. The latter effect resulted in better accessibility of ions such as Mg^{++} and Ca^{++} to the lipid phosphate groups. It is clear that these membranes are significantly different from those of non-halophiles, and a structural model has been developed as a basis for further studies. A second major area of investigation involves the light-induced effects in the purple membrane of halophilic bacteria. When cell envelope vesicles (containing purple membrane) are illuminated with yellow light, protons are ejected and an electrical potential develops across the membrane, as

determined with a fluorescent dye. These events were found to result in the uptake of several amino acids, an essential process in the cells. Detailed study showed that the carrier system apparently binds both the amino acid and sodium ion, and these are transported across the membrane together in response to the electrical potential. The importance of these findings is that, for the first time, an unambiguous system is available for study. The work will be continued by attempting to isolate the amino acid carrier proteins from the membranes. Contributing Personnel: J. K. Lanyi, W. Z. Plachy and F. Richardson (San Francisco State University), R. E. MacDonald (Cornell University), R. Renthal (NAS Associate), V. Yearwood-Drayton.

Oxygen Effects in Cells - The role of oxygen in modern organisms may be studied in one which can grow either with or without it. One such organism is the yeast Saccharomyces cerevisiae, which contains an enzyme (palmityl-coenzyme A-desaturase) that requires molecular oxygen for activity. This study has shown that the enzyme is present when the yeast is grown without oxygen, even though it has no apparent function under those conditions. Also the amount of enzyme can be increased by incubating the cells anaerobically with glucose;

thus oxygen is not necessary for the synthesis of this oxygen-requiring enzyme. When the enzyme was synthesized without oxygen, it was less stable, and dissociated into two portions which were inactive separately but active when recombined. In this case, the stability of the enzyme could be increased by growing the yeast in the presence of two other specific lipids. Apparently oxygen exerts an indirect effect on the stability of the enzyme, by allowing the synthesis of certain lipids which are essential for attachment of the enzyme complex to cellular membranes. The amount of oxygen actually necessary for enzyme activity is now being studied, using a very sensitive system which includes a gas chromatograph and oxygen electrode. There is little information on the oxygen concentration which is required for many such enzymes. The information is important in relation to the search for life on planets which have a very low level of atmospheric oxygen, and for consideration of early life forms on the primitive Earth, when oxygen concentrations were very low.

Another enzyme (acetyl-coenzyme A-synthetase) from the same yeast also shows oxygen effects. In this case, the organism makes two distinct enzyme proteins, depending on whether it is grown in limited or abundant oxygen supply. If

there is interference with oxygen utilization (by limiting oxygen supply, using inhibitors of aerobic metabolism, or making mutants with inactive mitochondria), only the non-aerobic enzyme is made. Because its physical properties are different, it is now being purified in order to compare it with the previously purified aerobic enzyme. Mutant strains of the yeast were prepared which were not capable of aerobic growth. These petite strains were capable of only anaerobic metabolism, and contained only anaerobic acetyl CoA synthetase. If two petites were crossed, however, a diploid wild type was obtained which could grow aerobically or anaerobically, and contained both forms of the enzyme. The genetics of the oxygen effect is being studied further.

Contributing Personnel: H. P. Klein, T. Satyanarayana (San Jose State University), C. Volkmann, L. Jahnke, N. Symmes.

In completing a study on one of the intermediates in the oxidative pathway, the oxidation-reduction potential of cytochrome c was determined. The potential was found to be identical whether determined in water or in deuterium oxide. This provides further support for the previously proposed water bridge theory for the deuterium isotope effect in intact cells.

Contributing Personnel: T. Kihara (NAS Associate), L. P. Zill.

Origin of Information Systems and Enzymes - Considerable evidence suggests that the evolution of the eucaryotic (nucleated) cell involved a fusion and subsequent symbiotic relationship between two or more primitive cell types. Various subcellular organelles, including mitochondria, are thought to represent remnants of this primitive event. Mitochondria have been shown to be semi-autonomous organelles with unique DNA and protein synthesizing systems which are separate from - yet integrated with - those of the balance of the cell. In studies in the yeast Saccharomyces cerevisiae, two independent procedures were developed for analyzing the timing of mitochondrial DNA replication, as well as the timing of mitochondrial gene replication. It was found that, whereas mitochondrial DNA is synthesized throughout the cell cycle, mitochondrial genetic information is most sensitive to mutagenic agents at a single period coinciding with the discontinuous synthesis of nuclear DNA. Additional work is planned to elucidate further details of this interrelationship.

Contributing Personnel: S. F. Cottrell (NAS Associate), and H. P. Klein.

One of the fundamental problems in the origin of life is understanding how the interactions between nucleic acids and amino acids led to development of the present information transfer systems of protein synthesis. An approach here is the study of the effect of temperature on the recognition of transfer-RNA and amino acids by the amino acyl-t-RNA synthetase enzymes. It is expected that the degree of association at different temperatures will indicate the type and strength of affinity involved. High temperatures will be especially important because they stress the system, and because one theory is that life began when the Earth was still hot. Both the enzyme and a specific transfer-RNA are now being purified from thermophilic and mesophilic organisms. A protein factor which stimulates the activity of the synthetase has been discovered and is being isolated. Another previous study on the effects of high temperature on enzyme kinetics will be extended to the use of physical techniques for characterizing changes in enzyme structure.

Contributing Personnel: M. Mazzotta (NAS Associate), R. D. MacElroy, R. Singleton (NAS Associate), R. Middaugh.

Collaborating Personnel: B. Strehler (University of Southern California), E. J. Johnson (Tulane University).

Before life appeared on the primitive Earth, there was probably catalytic activity which increased the rate of some of the reactions out of all of those possible. As a result, the formation of some compounds would be favored, so they and their catalysts would be more likely to find a place in organisms when living systems finally appeared. Because all biological catalysts (enzymes) are now proteins, it is logical to expect that the original catalysts were peptides. Several reports now support the theory by showing that peptides of only 4 or 5 amino acids show low orders of enzymatic activity. This work tested a number of peptides from various sources for "esterase" activity against p-nitrophenyl acetate. All peptides contained histidine because of its presence in the active site of the enzyme, and activities were compared with that of free histidine. The activity of histidine was enhanced in certain di-, penta-, hepta-, and undeca-peptides. Correlations are being made between their sequence and activity, and additional peptides will be obtained for testing.

Contributing Personnel: E. Bugna, M. R. Heinrich.

Collaborating Personnel: J. Young (University of California, Berkeley).

PROJECT STUDIES

Mars Surface Sample Return - Studies related to this proposed mission were continued, and a report prepared for Headquarters. Laboratory work further defined the minimum conditions required to sterilize soil, without destroying all the chemical information or the remaining biological structures. The study required the examination of several thousand petri plates prepared from soil samples heated under different conditions.

Heat-resistant Organisms - At the request of the Viking Biology Team Leader, the Biological Adaptation Branch (principal contributors: K. Souza and K. Shih) made an extensive study of a strain of very hardy spore-forming bacteria isolated at Kennedy Space Center. Determinations included survival curves at various temperatures and humidities, and characteristics of the survivors. Results were used to help establish the statistical constants required for Planetary Quarantine calculations. A meeting of NASA and contractor personnel engaged in determining planetary quarantine requirements for Viking was organized and held at Ames Research Center.

Mars Penetrator Study - This Branch participated in a study of biologically-significant experiments which had the potential for adaptation to a penetrator probe. Two areas were

identified which were very important to biology, and instrumental approaches were suggested. (1) Water is probably critical to the evolution of a native martian biota, to determining the probability for survival of terrestrial organisms which might contaminate the planet, and eventually for planned modification of Mars to make it habitable for humans. There is some evidence that the amount of water available in the soil is greater than would be expected from the concentration in the atmosphere. An experiment was proposed to determine water in the soil by a microwave probe mounted in the penetrator wall; an alternative is to use Nuclear Magnetic Resonance. Both types of equipment have been developed for soil measurements, and appear to be adaptable to penetrators. (2) The concentration of inorganic ions in the soil is important to biologists, because these ions are required for growth or to maintain structural integrity in bacteria. Knowledge of those concentrations would facilitate further life-detection missions to Mars, and would increase the probability that any organisms in a sample returned from Mars could be cultured successfully. In addition, the state of nitrogen (nitrate, nitrite, ammonia), sulfur, and other elements is important to planetologists and biologists. The proposed experiment would measure the concentration of several ions in an aqueous suspension of the soil, by means of ion-specific electrodes.

(3) An additional item of equipment which is under commercial development, and which may be useful in a variety of missions, is the miniaturized spectrophotometer. The complete instrument, including light sources, detectors, and electronics, has been reduced to about 3 cubic centimeters.

Planetary Engineering - The colonization of another planet has been suggested as an eventual necessity to solve the over-population problem on Earth. Even the most hospitable planet, Mars, would require major changes to make it habitable by humans. Biological Adaptation Branch personnel have started a study to delineate the parameters which would have to be controlled on Mars, and possible methods to attain the necessary conditions. Examples are the provision of water and an oxygen-containing atmosphere (possibly by photosynthetic organisms), and an increase in the mean temperature. Plans were made to set up a small study for summer 1975 by a joint Ames-American Society of Engineering Education group, followed by a larger design study in 1976. This program is being organized by R. D. MacElroy.

Extreme Environments Meeting - The third biennial meeting on this subject was held at Ames in June. The Branch organized and participated in the meeting, because of its responsibilities for work on microbial adaptation to environments existing on

other planets. Papers in the 2-1/2 day meeting stressed the mechanisms by which organisms adapt to high and low temperature, and high salt concentration. The papers will be published as a book.

PLANS FOR 1975

The Branch will continue its studies on the limits of life and the origin of life, and will expand its work in soil microbiology in relation to some projected missions.

The limiting conditions under which life can exist are determined by a number of biochemical factors. Some of the factors which will be studied include: (1) effects of ammonia in simulated Jovian atmospheres, and mechanisms of its toxicity; (2) the physical structure of membranes, transport systems, and other cellular components in organisms which can grow at high temperature or high salt concentrations; (3) the characteristics of organisms which can grow without oxygen, at low temperatures, and in high concentrations of salt.

The origin of life required the development of many different processes, and their gradual integration into a replicating system. Among the processes which will be studied in the laboratory are: (1) determination of the concentration of oxygen actually required for some oxidative enzymes, and

comparison with the levels of oxygen believed to be available on the primitive Earth; (2) study of the interaction of nucleotides and amino acids as the first step in developing an information transfer system, to be approached by means of computer modeling; (3) the catalytic activity of small peptides, which may represent primitive enzymes that increased their activity and specificity by the gradual addition of particular amino acids; (4) study of photosynthesis in high fluxes of ultraviolet light, as may have been present before the Earth developed its ozone layer.

The Biological Adaptation Branch expects to contribute to several studies and proposed missions. (1) The Branch has suggested experiments for a Mars Penetrator which would provide biologically-significant information. We will continue to develop background material for these experiments. If one is selected for a mission, the Branch will participate in development work and monitoring of the contract. (2) A Mars Surface Sample Return Mission has been proposed, and would offer the opportunity to study martian biology and chemistry in Earth laboratories. The Branch will continue a study on the relative value of sterilized vs. unsterilized soil samples, and methods of sterilization which do not destroy all the information in

the soil. The nature of the experiments which should be done on a martian sample, with a priority listing and a time schedule, will also be studied. This will lead to suggestions for design of a facility for absolute containment of the sample, but with provisions for carrying out all the necessary analyses. To prepare for this mission, there will be an increase in activities in the soil microbiology and biochemistry areas. (3) The cooperation with the Space Sciences Division will continue, particularly in providing them with information about the biological effects of ultraviolet light for their study of ozone layer changes, and about the tolerance of organisms for the Jovian atmosphere, for planetary quarantine calculations on the Jupiter probe.

LIFE DETECTION SYSTEMS BRANCH

Viking Gas Exchange Experiment

Test Standards Module for the Gas Exchange Experiment (GEX), one of three Viking Lander Biology Instruments, received attention from both TRW and ARC personnel throughout calendar year 1974. The test program included studies of the effects of flight constraints on the performance of the experiment and development of methods and of concepts or changes in sequencing to alleviate the problems exposed by the studies. The main emphasis was to consider any and all factors which could affect hardware design. Because valving could not be heated in the closed positions nor kept closed during the long transit time to Mars, column packing was found to be affected by the space vacuum. A successful method of regenerating the columns was invented and the conditions for this regeneration were determined. These are now incorporated in the flight experiment. An important inclusion to the experiment was invented to provide for a direct measure of nutrient injected. Neon gas was successfully incorporated into the nutrient ampoule and demonstrated to be an excellent choice for this diagnostic. The neon appears as a spike in the chromatogram before hydrogen gas. This concept did not alter the mechanical configuration of the flight instrument.

The above initiated concepts have been patent disclosure items initiated by V. Oyama. Active ARC participation in the TSM test program has been mainly B. Berdahl with support as needed from V. Oyama, G. Carle, P. Kirk and M. Lehwalt.

In-house tests with Q. A. support was provided by B. Berdahl in determining the M4 nutrient medium capability to support biological

activity after cruise stresses. Cruise stresses included 113°C for 140 hours followed by one year storage at 40°C. In addition, materials toxicity tests for nickel, various grades of stainless steel etc. showed no degradation of biological activity.

The adequacy of the flight sequence was tested on the TSM on an abbreviated schedule from the initialization to the final sterilization of the soil without significant incidence, demonstrating that no problems are inherent in the repeated actuations and events.

A new glass cell was designed by B. Berdahl and fabricated for laboratory use which allows an uninterrupted humidification cycle to be performed. Soil testing has begun using these cells and analyses are being performed based on the latest mission design.

All of the TSM operations were facilitated by the inclusion of a sequencing valve driver designed by G. Carle and fabricated under the direction of D. Humphrey (ARC-RFD). The valve driver allows automatic sequencing of the sample fill and inject routine and virtually eliminates human errors in sampling.

In preparation for flight readiness computer software provided by C. Reichwein (Martin Marietta) has been incorporated in the telecomputer facilities at the ARC and tested for adequacy. In addition, a relatively simple computer program requested by V. Oyama has been written by G. Carle that catalogs the GEX related laboratory data for use during Mars encounter. The program was constructed to take the raw, point by point data, determines the best preselected curve fit, characterizes it and stores it in a permanent data bank. The data bank can be recalled in its entirety and searched for corresponding GEX VLBI characteristics. This software allows the experimentor a choice of one or more selected data fits.

Terminal Heat Sterilization Survivors and Impact on the GEX Experiment

Fifty-one randomly selected "hardy" organisms from Kennedy Spacecraft Center were studied by M. Lehwalt to determine whether they would grow or survive under the conditions imposed by the Viking GEX should such an organism be deposited in the GEX biology package. When 3-30 spores of each culture were heated at 113°C for 60 hours under N_2 and incubated anaerobically in 2 ml of M4 medium, eleven of the 51 produced Colony Forming Units (CFU) when subcultured. These eleven isolates were used as inocula in gas exchange experiments and D values were also obtained.

Spore crops of the eleven test organisms selected for further study produced D values of 1.00 to 4.60 hours when tested at 113°C in a nitrogen environment. (Much higher D values were obtained with resistant spore crops produced by Dr. Campbell's group.)

Gas exchange experiments were run with the eleven isolates. 30-300 organisms in 2 ml M4 medium in glass cells containing an environment of 1.3% CO_2, 1.3% Kr, balance helium were incubated at room temperature for 16 days and analyzed at regular intervals by G. C. The only gas production was negligible amounts of N_2 and a small increase in CO_2. When the cell contents were plated aerobically and anaerobically eight of the isolates produced CFU aerobically and one of these isolates was facultative.

The gas exchange experiment was repeated using an incubation temperature of 15°C and two cycles (the first of 21 days and the second 26 days). There was negligible gas production and no colony forming units were obtained aerobically or anaerobically when the cell contents were plated at the end of each cycle. These experiments invalidate the concern that the GEX experiment will provide false positives from terrestrial contaminants.

Studies of the Gas Exchange Phenomena in GEX: Oxidation of Molecular Hydrogen by Anaerobic Soil Organisms

V. Oyama and B. Berdahl, while performing their Viking Gas Exchange experiments, repeatedly observed that some unknown microorganisms in a mixed soil population were able to utilize molecular hydrogen under anaerobic conditions. This phenomenon is rare; the ability to oxidize atmospheric hydrogen is limited to nitrifying, sulfate reducing, carbon dioxide reducing (in methane formation), and photosynthesizing bacteria. Because the hydrogen utilizing bacteria which they studied did not unequivocally fall into one of these categories, a study by H. Ginoza was initiated to see if these bacteria possessed an altogether different type of anerobic hydrogen-metabolism.

From a mixed population in Staten Island soil, two obligately anaerobic spore forming bacteria capable of utilizing molecular hydrogen were isolated by selectively growing them in the presence of a gas mixture initially containing 20% hydrogen, 2% carbon dioxide, and balance of helium. The organisms were 6-10 μ in length, gram variable, actively motile and at times formed short chains. Their nutritional requirements are still undefined but growth is good when soil is added to nutrient media.

Molecular hydrogen is efficiently utilized by the spore forming bacteria in the presence of soil particles but is considerable reduced in its absence. Soil can be replaced by an unknown factor found in tomato juice. The process occurs over a wide temperature range, 26-38°C. The organisms grow optimally between pH of 7.8 and 8.7 but hydrogen uptake occurs over a much broader range, 6.5 to 9.0.

Hydrogen uptake normally starts after growing bacteria reach the stationary phase. Growth usually stops after 3-5 days of incubation,

however, hydrogen utilization continues indefinitely until most of the hydrogen is exhausted. Consumption of hydrogen was even noticeable when vegetative cells had completely sporulated.

Some preliminary studies suggest that these organisms are indeed not denitrifying, sulfate reducing, methane producing or photosynthesizing bacteria. However, they have some features which are closely associated with Clostridium aceticum, a unique bacteria which was originally reported 35 years ago, to be able to derive all its energy for growth from oxidation of hydrogen and reduction of carbon dioxide.

The study is still far from completion. The fate of the oxidized hydrogen molecule is still unknown. The relationship, if any, between hydrogen utilization and spore formation is interesting but still speculative. The possibility still exists that the organism can subsist on energy derived from oxidation of hydrogen and reduction of carbon dioxide.

Studies of the Gas Exchange Phenomena in GEX: Carbon Monoxide Metabolism

In addition to the hydrogen consumption aspects, carbon monoxide metabolism is a likely phenomenon that may occur in the Martian environment. B. Berdahl and V. Oyama reported earlier on the basic anaerobic aspects of this metabolism. Of the inhibitors, azide inhibited both oxygen and carbon monoxide uptake totally at 10^{-2} M, while cyanide at 10^{-2} M and antimycin A at 10^{-4} M did not.

A general test of soils collected from various parts of the world showed that water or the GEX medium (M4) sufficed to demonstrate CO uptake. A direct correlation of CO uptake with factors which produce anaerobic conditions in soils is suggested, thus pointing up the possibility that CO metabolism may well be functioning in the Martian environment.

Pioneer Venus Gas Chromatograph Atmospheric Analyzer (PVGCAA)

The PVGCAA was accepted by NASA Headquarters for use in the large probe in the study of the atmosphere of Venus. Also during this year, the initial test program was completed, the breadboard was refurbished and the final breadboard tests begun. These tests are being performed by G. Carle and P. Kirk and will define more clearly such areas as sensitivity, instrument sequence, retention time internal standards injection as well as S/C environmental effects on the instrument as a whole.

In the area of chromatograph column packing development, F. Woeller with support from W. Christensen (San Jose State Work Study Student) has defined the required specifications for monomers and solvents used in the polymerization of styrene and divinylbenzene. Specific polymerization routes including reactant and solution strengths as well as shaping of polymers are in study to characterize product properties necessary to achieve the optimum separations for the PVGCAA.

F. Woeller and G. Carle attended a meeting with John Hoffman (Principal Investigator for the PV Mass Spectrometer) and his coworkers and arrived at a preliminary agreement to cooperate in the development of both the gas chromatograph and the mass spectrometer. Areas of interest to both groups were testing of both instruments in the same environment at the same time, sharing and trading of test standard gas mixtures and the design and fabrication of a Venus atmospheric simulator. The primary goal of such an agreement was to attempt to insure a complete and accurate analysis of the atmosphere of Venus.

In the laboratory effort, F. Woeller with substantial contractual support is solving a considerably difficult problem of qualifying the PVGCAA concept for the Venus atmospheric sampling and analysis. The

chromatographic problem is mainly in the expectation that the Venus atmosphere will contain strongly acidic constituents such as hydrogen chloride, sulfuric acid, hydrogen bromide and hydrogen fluoride. These molecules chemically react with metallic materials generally used in the construction of chromatographic gas flow paths. A novel test bench (at the suggestion of V. Oyama) has been constructed to allow for a comprehensive testing of various parts and of materials. Preliminary results show that the chromatographic process is the most susceptible to reactive constituents because the mass action phenomenon and renewed surfaces in the chromatographic process are not amenable to quantitation. Now that a method is available to assess sorptive phenomena, the problem of ascertaining responsible agents is resolved. It is a matter now of using materials which do not react or developing methods by which nonreplaceable parts having reactive surfaces are coated with inert materials.

Outer Planet Studies

The ARC Outer Planet team funded and received a feasibility study of gas chromatograph concepts for analyzing the composition of the atmosphere of Uranus with particular emphasis on measuring the hydrogen and helium ratio. The study lasted a few months and was reported in two small volumes and delivered December 17, 1974. The first part of this study was performed by the in-house complement of F. Woeller, V. Oyama and G. Carle and the second part was performed by TRW under NAS 2-7867. The in-house report dealt mainly with a study of the gas chromatographic configurations and showed by laboratory tests that the hydrogen/helium ratios could be attained by the use of neon carrier gas and a relatively insensitive operation mode of thermistor detectors. Neon was determined to be the most likely candidate

over nitrogen and argon, because it provided exact linear responses over the range from 0.1% to 100% for the two gases. The added feature of ionization voltage (near helium) permits it to be used effectively with ionization detectors for most gases (except neon) including all the likely candidates expected to be in the hydrogen rich outer planets.

The engineering aspects covered weight, power and volume, as well as environmental aspects of the various options posed by the in-house study.

Advanced Life Detection Aspects: Metabolic Activity Measured by Electrical Conductivity

In previous studies aimed at detecting life, M. P. Silverman and E. F. Munoz showed that in 12 different soils amended with a glucose solution the electrical conductivity and a number of water soluble inorganics increased with time as a function of glucose metabolism by the native soil microorganisms. It has now been shown that increases in electrical conductivity and water soluble calcium and magnesium always occur in all the soils tested to date with glucose under anaerobic incubation. These three parameters always increase for the first 7 to 10 days and maintain their maximum levels for the duration of the 14-day experiments. In addition, there is always an increase in acidity which follows the same kinetics. In contrast, these same experiments run aerobically showed an increase in electrical conductivity, water soluble calcium and magnesium, and acidity which generally peaked in 3 to 7 days and then declined for the remainder of the experiment. Among the inorganics tested the most reliable for use as indicators of metabolic activity were calcium and/or magnesium, potassium, sodium in that order. Other anerobic experiments were run with Aiken, Hesperia and Waukena soils amended with the M4 medium used in the Viking

GEX experiment. This medium is rich in organic compounds and salts and is very high in electrical conductivity. Nevertheless, positive indications of metabolic activity were obtained in these soils from water soluble Mg measurements, from water soluble Ca measurements in Aiken and Waukena soils, and from electrical conductivity measurements in Hesperia and Waukena. Therefore, detection of extraterrestrial metabolism of soils by means of electrical conductivity measurements and water soluble Ca and Mg measurements continue to show promise.

Biological Characterization: Dye Fluorescent-DNA Complex

Further work on the characterization of microorganisms based on energy derived from fluorescent dye-DNA complex has shown that double stranded polynucleotide in most terrestrial bacteria can be measured quantitatively and specifically with various acridine and phenanthredene dyes.

Ethidium bromide, a highly fluorescent dye, was found to form a strong dye-DNA complex with most bacterial DNA and RNA. In the complexed state, the fluorescent yield of the dye was always enhanced by one or two orders of magnitude. In addition, the excitation peak of the fluorescent molecule was red-shifted from 490 to 515 nm. If the dye-DNA complex was heated to 90-100°C, the bound structure was disrupted, resulting in return of the original excitation pattern and also a corresponding decrease in fluorescence. The shift of excitation peak and enhancement of fluorescent energy yield by the dye-DNA complex is a useful tool for characterization of extra-terrestrial micororganisms.

Phospholipids: Integrity of Membranes and Thermophily

L. Kostiw's contributions on the effects of temperature on the phospholipids of a thermophile and its temperature-sensitive mutant has resulted in

additional insights into the problem of the mutant's inability to grow at high temperature (65°C). Comparison of the synthesis and turnover of the phospholipids of both strains showed that the cardiolipin (CL) and phosphatidylglycerol (PG) content could play a part in the integrity of the mutant cell membrane.

When incubated under conditions which permitted no net synthesis of phospholipids, but at 65°C, the phospholipid composition of the mutant changed markedly; CL increased proportionally to the decrease in PG, resulting in a CL/PG ratio of 9.5 just prior to the onset of lysis. Conversion of PG to CL occurred also in the parent, but the ratio of CL/PG was only 4.8. In addition, the mutant accumulated lysoCL (tentative identification), a hydrolysis product of CL. It appears that a higher incubation temperature causes a distortion in the PG <--> cycle and stimulates hydrolysis of CL. Since bacteria increase their CL content under stress conditions, the mutant's reaction to growth at higher temperature can be interpreted as correct. However, the reaction also results in the accumulation of a hydrolytic CL product, besides being an overreaction in comparison to the parent. Both factors might affect the corrective response and contribute to lysis.

When incubated in the presence of chloramphenicol at 65°C, the mutant does not lyse. Phospholipid synthesis takes place in both the parent and the mutant, but at a reduced rate. CL synthesis from PG occurs in both strains at similar rates. The CL/PG ratio in both strains is similar until 36 minutes of incubation. At that time, the parent achieves a ratio of 0.9, and the mutant a ratio of 0.46. The amount of lysoCL in both is similar and does not increase significantly. It appears that chloramphenicol prevents excess synthesis of CL from PG and hydrolysis of CL by inhibiting

either increased synthesis or activities of the enzymes involved. Since CL and PG synthesis proceeds at similar rates in the parent and the mutant, the amount of anionic phospholipids (CL and PG) remains relatively stable in relation to the amount of the neutral phospholipid, phosphatidylethanolamine. Thus the balance between the differently charged membrane components is preserved and contributes to the integrity of the cell membrane of the mutant.

Phospholipid Assay

Another project undertaken in 1974 was the development of a method for the assay of intact phospholipid molecules. From 10-30 nmoles of phospholipid can be detected by this method. The method depends on complex-formation between the phospholipid and molybdenum, in the presence of strong acid. The complex is extracted with chloroform and assayed spectrophotometrically. Refinement of the method is still needed, however.

Mars Sample Return: Methods for Sample Sterilization

A soil sterilization treatment using mineral acids at ambient temperature was evaluated by E. Merek and O. Whitfield for possible use for a returned Martian sample. The treatment of a variety of soils with 1 normal hydrochloric acid resulted in sterilization. Bacterial vegetative cells and spores (pure cultures) were killed in hours by this treatment, and the survival curves have the same predictability as those obtained by dry heat treatment. Bacterial cells retained their initial morphological appearance and were still recognizable after this treatment. With this type of sterilization, changes in organic composition of a sample should be less than that resulting from any heat treatment and any changes should be more predictable.

Water Analysis

P. Kirk and G. Carle in their continuing study of extraction methods for water in soils preparatory to gas chromatographic separation and analysis of the solution, have demonstrated the feasibility of multiple extraction procedures. The total water extracted from sequential extractions from a single sample using methylal followed by methanol yields the same water as if a single extraction were performed with methanol (the stronger solvent) upon a given sample. Techniques utilizing multiple extractions may well be applicable to soil water analysis.

In another aspect more related to the biological interest, E. Merek has found that benzene solvent extraction more nearly relates to the water activity of soil, i.e. parallels the relative humidity of the soil sample.

Other Participations

An increasing rapport with Space Sciences and Development groups is evolving with the aim of better collaboration towards planetary atmospheres research and the understanding of the Earth's own atmosphere. F. Woeller provided analytical support to wind-tunnel experiments with Space Shuttle models in which a gas chromatograph device was used to follow wind tunnel gas dynamics.

In collaboration with outer planet entry probe team, F. Woeller participated in determining how probe shield impacts gas sampling for gas chromatograph and mass spectrometry by monitoring the simulation of outgassing in wind tunnel experiments using a gas chromatograph modified to measure Freons.

B. Tyson has provided support to Space Sciences in determining whether space developed concepts for measuring organics in the atmosphere could be applied to the current interest in Freons. He has verified by mass spectral means in the parts per trillion level the presence of Freon 11 and 12 in

the atmosphere, hitherto assumed to be present by GC electron capture techniques. He has further determined the presence of Freon 113 (a widely used industrial cleaner) in the Santa Clara Valley. The prescence of this Freon has not been previously reported. The general applicability of extending these tasks to organics in the atmosphere in general has been appreciated by the "New Initiatives Program" in the acceptance of the proposal "Detection of Organic Compounds in the Air" as one of four to be sponsored by Langley.

EXPECTATIONS FOR CALENDAR YEAR 1975

In August 1975 it is expected that Viking Mission A will be launched followed a few months later by B. For that preparation a readiness review will have occurred both in the science and engineering areas. The science aspects will have authenticated the correlation of laboratory and flight instrument test data. Included in these tasks will be the calibration of nonstandard test gases, determination of correction coefficients for gas solubilities and tests validating the software to store as well as to retrieve data. In addition, during the cruise phase the TSM will be used to establish the characteristics on data resulting from singular failure modes.

The Pioneer Venus procurement contract will be expected to be promulgated and work will proceed well on the way to flight design and fabrication. In-house studies on flight performance specification and chromatographic column packing development should be completed in time for actual input into the design and function of the gas chromatograph. Simulations of the descent environmental profile should provide a better estimate of accuracy and precision capability.

In-house laboratory study on the hypothesis that heat resistant spores do not germinate under anaerobic conditions will be tested for a number of heat treated soils while germination of these same organisms will be tested under aerobic conditions. Subtasks on the hydrogen consuming organisms and carbon monoxide metabolism will be continued.

New starts for calendar year 1975 will include a definitive study on penetrator gas analyzer concepts to detect life and a 1974 proposal solicited by EPA, NEAC in Corvallis, Oregon entitled "A Preliminary Proposal to Determine

the Nature and Levels of Hydrocarbons Emitted from Major Vegetation Types in the Western United States" may very well be implemented in 1975. In addition a proposal for developing a direct method for estimating viral populations in waters, may well be approved by the Office of Applications. This work is an outgrowth of H. Ginoza's effort on the dye-DNA/RNA fluorescence technique.

It is expected that outer planet work on the gas chromatographic aspects will touch upon new concepts of column capability including developing in situ polymerization techniques for capillary columns and detector characterization for identification purposes.

In the area of electrical conductivity, future work will entail the evaluation of a commercial instrument containing sample cuvette with built-in electrodes and capable of continuously monitoring electrical conductivity changes in 32 samples run simultaneously. The feasibility of using ion specific electrodes to measure water soluble Ca and Mg changes during metabolism will also be evaluated, as well as a variety of different substrates to elicit metabolic activity.

Phospholipids studies will be limited to subjects which will complete the research project to the state in which manuscripts can be finalized and include verification of the structure of lysoCL, alteration of polar head groups in bacterial lipids and refinement of the intact phospholipid assay.

PLANETARY BIOLOGY DIVISION

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1974 PUBLICATIONS

LIFE DETECTION SYSTEMS BRANCH

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PLANETARY BIOLOGY DIVISION
LECTURES, SEMINARS AND PRESENTATIONS AT SCIENTIFIC MEETINGS

1974

Lectures & Seminars
Chemical Evolution Branch
1974

January 17	De Anza College Cupertino, CA	Meteorites and Organic Cosmochemistry (Keith A. Kvenvolden)
January 28	Stanford University Stanford, CA	Meteorites and the Moon (Carleton B. Moore)
February 14	San Jose State Univ. San Jose, CA	Chemical Evolution (Keith A. Kvenvolden)
February 26	Rickey's Hyatt House Palo Alto, CA	Rocks from the Moon (Carleton B. Moore)
February 28	San Francisco State San Francisco, CA	Chemical Evolution in Planetary Atmospheres (Sherwood Chang)
March 7	San Francisco State San Francisco, CA	Organic Chemistry of Meteorites (James G. Lawless)
March 14	San Francisco State San Francisco, CA	Origin and Early Evolution of Life on Earth (Keith A. Kvenvolden)
March 21 & April 30	San Jose State Univ. San Jose, CA	Early Life on Earth (Keith A. Kvenvolden)
April 11	University of Delaware Newark, Delaware	Geochemical Evidence for Chemical and Early Biological Evolution (Keith A. Kvenvolden)
April 16	Univ. of Maryland Maryland	Precambrian Organic Geochemistry (Keith A. Kvenvolden)
April 25	De Anza College Cupertino, CA	Evolution of Early Life (Keith A. Kvenvolden)

April 26	University of Montana Montana	Evidence for Chemical and Early Biological Evolution (Keith A. Kvenvolden)
May 6	Archbishop Mitty H.S. San Jose, CA	Chemical Origin of Life (Sherwood Chang)
May 22	Mt. Diablo High School Concord, CA	Chemist in Life Sciences (James G. Lawless)
May 22	Concord High School Concord, CA	Chemical Evolution Research (James G. Lawless)
June 1	Lafayette College Easton, PA	Outer Space-Fact and Fantasy (James G. Lawless)
July 16	Ames Research Center Moffett Field, CA	Meteorites and Organic Cosmochemistry (Keith A. Kvenvolden)
September 6	Chemistry Club, U.S. Air Force, Ames Research Center Moffett Field, CA	Organic Chemistry in Space (Keith A. Kvenvolden)
October 3	DeAnza College Cupertino, CA	Life on Mars (Keith A. Kvenvolden)
October 3	Department of Chemistry Kansas State University Manhattan, KS	Organic Cosmochemistry: Evidence from Meteorite Analysis (James G. Lawless)
October 15	Speakers Club, University of California, Santa Barbara, CA	Evidence for Chemical Evolution from Carbonaceous Meteorites and the Early Earth (Keith A. Kvenvolden)
October 16	Paleobiogeology Group University of California Santa Barbara, CA	Advances in the Geochemistry of Amino Acids (Keith A. Kvenvolden)

Technical Meeting Presentations
Chemical Evolution Branch
1974

March 19	5th Lunar Science Conference The Lunar Science Institute Houston, TX	Abundances of C, N, H, He, and S in Apollo 17 soils from stations 3 and 4: implications for solar wind exposure ages and regolith evolution (Sherwood Chang)
April 9	55th Annual American Geophysical Union Meeting Sheraton-Park Hotel Washington, D.C.	Amino and Fatty Acids in Murchison and Murray Meteorites (Keith A. Kvenvolden)
May 22	Mass Spectrometry meeting Philadelphia, PA	GC-MS in the Analysis of Dicarboxylic Acids (James G. Lawless)
August 7	37th Annual Meteoritical Society Meeting Los Angeles, CA	Recent Results from Studies of Organic Acids in the Murchison Carbonaceous Chondrite (James G. Lawless)
August 30	Gordon Research Conference on Organic Geochemistry Plymouth, New Hampshire	Amino and Fatty Acids in Geo- and Cosmochemistry (Keith A. Kvenvolden)
September 10	1974 American Chemical Society Meeting, Convention Hall, Atlantic City, NJ	Solar Wind Influences on the Chemistry of C,N,H,S, and Metallic Fe in Lunar Soils (Sherwood Chang)
October 18	1974 Pacific Conference on Chemistry and Spectroscopy, Jack Tar Hotel, San Francisco, CA	Thermal Diagenesis of Amino Acids in Meteorites, Rocks, and Shells (Etta Peterson)
October 18	1974 Pacific Conference on Chemistry and Spectroscopy, Jack Tar Hotel, San Francisco, CA	Dicarboxylic Acids in Meteorite and Simulated Chemical Evolution Samples (Ben Zeitman)

December 13	American Geophysical Union Meeting, Jack Tar Hotel, San Francisco, CA	Lunar Surface Chemistry of Solar Wind Implanted Carbon, Nitrogen and Hydrogen Species (Sherwood Chang)
December 16	American Geophysical Union Meeting, Jack Tar Hotel San Francisco, CA	Amino-Acid Enantiomeric Ratios as Correlative and Chronological Tools in the Study of California Marine Terraces (John Wehmiller)
December 16	American Geophysical Union Meeting, Jack Tar Hotel, San Francisco, CA	Effects of Sterilization Procedures on Samples Returned from Mars (Keith A. Kvenvolden)

BIOLOGICAL ADAPTATION BRANCH

LECTURES, SEMINARS AND PRESENTATIONS AT SCIENTIFIC MEETINGS

Organization	Title/Speaker
Instituto de Investigaciones Bioquimicas Universidad de Buenos Aires Buenos Aires, Argentina	Light Energy Transduction by the Purple Membrane of H. halobium. (Roberto Bogomolni)
Universidad de Buenos Aires Facultad de Medicina Buenos Aires, Argentina	A New Light Energy Transducer: The Purple Membrane of H. halobium. (Roberto Bogomolni)
College Park Colloquium on the Chemical Evolution of the Giant Planets College Park, Maryland	High pH, Ammonia Toxicity, and the Search for Life on the Jovian Planets. (Paul H. Deal)
Division of Biological Sciences San Francisco State University San Francisco, California	Terrestrial Bacteria as Models for Life on Other Planets. (Milton R. Heinrich)
Space Biology Student Program NASA-Ames Research Center Moffett Field, California	Studies of Extreme Environments by the Biological Adaptation Branch. (Milton R. Heinrich)
Department of Biological Sciences San Francisco State University San Francisco, California	Halophilic Bacteria. (Lawrence I. Hochstein)
National Institute of Dental Research National Institutes of Health Bethesda, Maryland	Halophilic NADH-Dehydrogenases. (Lawrence I. Hochstein)
Extreme Environments Meeting NASA-Ames Research Center Moffett Field, California	Studies on the Activity and Stability of Halophilic NADH-Dehydrogenases. (Lawrence I. Hochstein)
Biophysical Soc. - American Soc. of Biol. Chem. Meeting Minneapolis, Minnesota	Bilayer Structure in Vesicles of Halobacterium cutirubrum Lipids. (Janos K. Lanyi)

Organization	Title/Speaker
Cardiovascular Research Institute University of California San Francisco, California	Light Energy Conversion by H. halobium. (Richard Lozier)
Carnegie Institution Stanford, California	Photochemical Reaction Cycle of Bacteriorhodopsin. (Richard Lozier)
Chemical Biodynamics Laboratory University of California Berkeley, California	Light Induced Optical Absorbance Changes in Chloroplasts at 77°K. (Richard Lozier)
Department of Chemistry University of Paris Creteil, France	Life on Jupiter - A Possibility. (Robert D. MacElroy)
Department of Microbiology University of Amsterdam Amsterdam, Holland	Thermal Behavior of a Thermophilic Enzyme. (Robert D. MacElroy)
Department of Microbiology University of Groningen Groningen, Holland	Some Ideas on Jovian Life. (Robert D. MacElroy)
Department of Chemistry Rensselaer Polytechnic Inst. Troy, New York	Life on the Outer Planets. (Robert D. MacElroy)
Department of Chemistry University of Maryland College Park, Maryland	Life on the Second Sun. (Robert D. MacElroy)
COSPAR Meeting Sao Paulo, Brazil	Biology on the Outer Planets. (R. S. Young & R. D. MacElroy)

Organization	Title/Speaker
Children's Hospital Oakland, California	Regulatory and Enzymological Aspects of Acetyl-CoA Synthetase of Yeast. (T. Satyanarayana)
Indian Institute of Science Bangalore-12, India	Two Acetyl-CoA Synthetases of Yeast. (T. Satyanarayana)
Christian Medical College Hospital Vellore, India	Studies on Acetyl-CoA Synthetases of S. cerevisiae. (T. Satyanarayana)
Department of Chemistry California State University San Jose, California	Enzymology and Advanced Isolation Methods. (T. Satyanarayana)
Department of Biology San Francisco State University San Francisco, California	Characteristics of Thermophilic Organisms. (Kenneth A. Souza)
West Coast Bacterial Physiologists Annual Asilomar Conference Pacific Grove, California	Temperature Effects on the Membrane of a Thermophilic Bacillus. (Kenneth A. Souza)
Extreme Environments Meeting NASA-Ames Research Center Moffett Field, California	Growth Temperature and Structure of the Lipid Phase. (A. F. Esser & K. A. Souza)
COSPAR Meeting Sao Paulo, Brazil	Problems with Returned Mars Samples (R. S. Young and D. L. DeVincenzi)
Career Day Cupertino High School Cupertino, California	Research in NASA Life Sciences (Donald L. DeVincenzi)
Space Biology Student Program NASA-Ames Research Center Moffett Field, California	Research in Planetary Biology (Donald L. DeVincenzi)
Peninsula Harvard Club Stanford University Stanford, California	The Search for Life on Mars (Donald L. DeVincenzi)

PLANETARY BIOLOGY DIVISION

PROFESSIONAL ACTIVITIES

1974

Professional Appointments and Offices

Chemical Evolution Branch

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I. University Faculty	
Keith A. Kvenvolden	Consulting Professor (Geology) Stanford University
James G. Lawless	Visiting Assistant Professor (Chemistry) University of Santa Clara
II. NASA Committees	
Sherwood Chang	Lunar Sample Analysis Planning Team Lunar Sample Curators Facilities Working Group
James G. Lawless	Advisor - Instrument Review Committee, Pioneer-Venus
III. NASA Appointments	
Sherwood Chang	Principal Investigator, Lunar Sample Program
IV. Officers of Professional Societies	
Keith A. Kvenvolden	Councilor, Geochemical Society Chairman, Commission on Organic Geochemistry, International Association of Geochemistry and Cosmochemistry
V. Editorships	
Keith A. Kvenvolden	Associate Editor, Geochimica et Cosmochimica Acta Advisory Editor, Biosystems Editorial Review Board, Colorado School of Mines

VI. Committees

Keith A. Kvenvolden	Chairman, JOIDES Advisory Panel on Organic Geochemistry Member, Scientific Visiting Committee for Chemistry, Woods Hole Oceanographic Institution

VII. Honors

James G. Lawless	Distinguished Alumni Lecturer, Department of Chemistry, Kansas State University

PROFESSIONAL ACTIVITIES

M. R. Heinrich	Organized 3rd Biennial Meeting, "Extreme Environments: Mechanisms of Microbial Adaptation," Ames Research Center, June 1974.
L. I. Hochstein	Program Chairman, West Coast Bacterial Physiology Annual Asilomar Conference, December 1974.
R. D. MacElroy	Vice-Chairman and Acting Chairman, Committee on Environmental Microbiology, American Society for Microbiology.
K. A. Souza	Organized Working Conference on Hardy Organisms, Ames Research Center, November 1974.
L. P. Zill	Ames Research Center Representative, Research Council of the Office of Aeronautics and Space Technology, NASA Headquarters.
	Administrator, Ames-University Consortium.
D. L. DeVincenzi	Chairman, Life Sciences Academic Affairs Committee